



#6

09892613.ST25

SEQUENCE LISTING

<110> Leung, Shawn Shui-on

<120> REDUCING IMMUNOGENICITIES OF IMMUNOGLOBULINS BY FRAMEWORK-PATCHING

<130> 655

<140> US 09/892,613

<141> 2001-06-27

<160> 32

<170> PatentIn version 3.1

<210> 1

<211> 369

<212> DNA

<213> Artificial Sequence

<220>

<223> FR-patched heavy chain variable region sequence (Full DNA Sequence) formed by joining the N- and C- terminal (SEQ 3 and 6) halves at the KpeI site.

<220>

<221> V\_region

<222> (1)..(369)

<223>

<400> 1

gaagtgcagc	tgctggagtc	tgggggaggc	ttagtgcagc	ctggagggtc	cctgaggctc	60
tcctgtgcag	cctctggatt	ctccttcagt	atctatgaca	tgtcttgggt	tcgccaggca	120
ccgggaaagg	ggctggagtg	ggtcgcatac	attagtagtg	gtggtggtac	cacctactat	180

## 09892613.ST25

ccagacactg tgaagggccg attcaccatc tccagagaca atgccaagaa ctccctgtac 240  
 ctgcaaata acagtctgag ggtggaggac acagccttat attactgtgc aagacatagt 300  
 ggctacggtg gtagctacgg ggttttgttt gcttactggg gccaaaggac tctggtcact 360  
 gtctctttca 369

<210> 2

<211> 123

<212> PRT

<213> Chimaera sp.

<400> 2

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Ser Ile Tyr  
 20 25 30

Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45

Ala Tyr Ile Ser Ser Gly Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Val  
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr  
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Val Glu Asp Thr Ala Leu Tyr Tyr Cys  
 85 90 95

Ala Arg His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr  
 100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
 115 120

<210> 3

<211> 111

<212> DNA

<213> Artificial Sequence

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<220>

<223> N-template is a synthetic sense-strand oligonucleotide encoding a  
mino acids 14-50 of the VH region (SEQ ID No. 2). The template is  
PCR-amplified by two primers (SEQ ID No. 4 and 5)

<220>

<221> V\_region

<222> (1)..(111)

<223>

<400> 3  
cctggagggg ctctgaggct ctctgtgca gcctctggat tctccttcag tatctatgac 60  
atgtcttggg ttccgaggc accgggaaag gggctggagt gggtcgcata c 111

<210> 4

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding am  
ino acid 1-19 of the VH region (SEQ ID No. 2). The 3' end of the  
primer overlaps with the 5' end of the template by 18 nucleotides

<220>

<221> primer\_bind

<222> (1)..(57)

<223>

<400> 4  
gaagtgcagc tgctggagtc tgggggaggc ttagtgcagc ctggaggggc cctgagg 57

<210> 5

<211> 48

<212> DNA

<213> Artificial Sequence

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<220>

<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 43-59 of the VH region (SEQ ID No. 2). The primer overlaps with the template by 21 nucleotides.

<220>

<221> primer\_bind

<222> (1)..(48)

<223>

<400> 5  
gtaggtggta ccaccaccac tactaatgta tgcgaccac tccagccc 48

<210> 6

<211> 132

<212> DNA

<213> Artificial Sequence

<220>

<223> C-terminal is a synthetic sense-strand oligonucleotide encoding amino acid 68-111 of the VH region (SEQ ID No 2) The template is PCR-amplified by two primers (SEQ ID No 7 and 8)

<220>

<221> V\_region

<222> (1)..(132)

<223>

<400> 6  
ttcaccatct ccagagacaa tgccaagaac tcctgtacc tgcaaatgaa cagtctgagg 60  
gtggaggaca cagccttata ttactgtgca agacatagtg gctacggtag tagctacggg 120  
gttttggttg ct 132

<210> 7

<211> 60

<212> DNA

<213> Artificial Sequence

&lt;220&gt;

<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 55-74 of the VH region (SEQ ID No 2). The 3' end of the primer overlaps with the 5' end of the template by 21 nucleotides

&lt;220&gt;

&lt;221&gt; primer\_bind

&lt;222&gt; (1)..(60)

&lt;223&gt;

<400> 7  
 ggtggtacca cctactatcc agacactgtg aagggccgat tcaccatctc cagagacaat 60

&lt;210&gt; 8

&lt;211&gt; 57

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 105-123 of the VH region (SEQ ID No 2). The primer and the template overlaps by 21 nucleotides.

&lt;220&gt;

&lt;221&gt; primer\_bind

&lt;222&gt; (1)..(57)

&lt;223&gt;

<400> 8  
 tgaagagaca gtgaccagag tcccttgGCC ccagtaagca aacaaaaccc cgtagct 57

&lt;210&gt; 9

&lt;211&gt; 321

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

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<223> FR-patched light chain variable region sequence formed by joining the N- and C- terminal (SEQ 11 and 14) halves at the KpeI site.

<220>

<221> V\_region

<222> (1)..(321)

<223>

<400> 9  
gatatccaga tgacccagtc tccatcctcc ctgtctgcct ctgtgggaga cagagtcacc 60  
attagttgca gggcaagtca ggacattagc aattatttaa actggtatca gcagaaacca 120  
ggtaaggctc cgaaactcct gatctactac actagtatat tacactcagg agtcccatca 180  
aggttcagtg gcagtgggtc tggaacagaa tttactctca ccattagctc cctgcagcca 240  
gaagattttg ccacttactt ttgccaacag ggtaatacgc ttccgtggac gttcgggtgga 300  
ggcaccaagg tggaatcaa a 321

<210> 10

<211> 107

<212> PRT

<213> Chimaera sp.

<400> 10

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr  
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp  
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
           100                                  105

<210> 11

<211> 108

<212> DNA

<213> Artificial Sequence

<220>

<223> N-template is a synthetic sense-strand oligonucleotide encoding a  
 amino acid 11-46 of the VL region (SEQ ID No. 10). The template is  
 PCR-amplified by two primers (SEQ ID No. 12 and 13)

<220>

<221> V\_region

<222> (1)..(108)

<223>

<400> 11

ctgtctgcct ctgtgggaga cagagtcacc attagttagca gggcaagtca ggacattagc 60  
 aattatttaa actggtatca gcagaaacca ggtaaggctc cgaaactc 108

<210> 12

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding am  
 ino acid 1-17 of the VH region (SEQ ID No 10). The 3' end of the  
 primer overlaps with the 5'end of the template by 21 nucleotides

<220>

<221> primer\_bind

<222> (1)..(51)

<223>

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<400> 12  
gatatccaga tgaccagtc tccatcctcc ctgtctgcct ctgtgggaga c 51

<210> 13

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 40-53. The primer and the template overlaps by 18 nucleotides.

<220>

<221> primer\_bind

<222> (1)..(40)

<223>

<400> 13  
atatactagt gtagtagatc aggagtttcg gaggccttacc 40

<210> 14

<211> 120

<212> DNA

<213> Artificial Sequence

<220>

<223> C-terminal is a synthetic sense-strand oligonucleotide encoding amino acid 59-98 of the VH region (SEQ ID No 10) The template is PCR-amplified by two primers (SEQ ID No 15 and 16)

<220>

<221> V\_region

<222> (1)..(120)

<223>

<400> 14  
ccatcaaggt tcagtggcag tgggtctgga acagaattta ctctcaccat tagctccctg 60



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cagccagaag attttgccac ttacttttgc caacagggta atacgcttcc gtggacgttc 120

<210> 15

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 50-65 of the VH region (SEQ ID No. 10). The 3' end of the primer overlaps with the 5' end of the template by 21 nucleotides

<220>

<221> primer\_bind

<222> (1)..(49)

<223>

<400> 15

ctacactagt atattacact caggagtccc atcaaggttc agtggcagt 49

<210> 16

<211> 48

<212> DNA

<213> Artificial Sequence

<220>

<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 92-107 of the VH region (SEQ ID No 10). The primer and the template overlaps by 21 nucleotides.

<220>

<221> primer\_bind

<222> (1)..(48)

<223>

<400> 16

tttgatttcc accttggtgc ctccaccgaa cgtccacgga agcgtatt 48

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<210> 17  
 <211> 371  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> FR-patched heavy chain variable region sequence (Full DNA Sequence) formed by joining the N- and C- terminal (SEQ 19 and 22) halves at the KpeI site.  
 <220>  
 <221> V\_region  
 <222> (1)..(371)  
 <223>

<400> 17  
 caggtgcaac tgggtggcttc cggggctgag gtaaataagc ctggggcctc agtgaaggctc 60  
 tcctgcaagg cttctggcta cacatttacc agttacaata tgcactgggt acggcagcct 120  
 cctggaaggg gcctggaatg gattggagct atttatccag gaaatgggtga tactagttac 180  
 aatcagaaat tcaagggcaa ggccacattg actgcagaca aatcctccag cacagcctac 240  
 atgcagctca gcagtctgac atctgaggac tctgcggtct attactgtgc aagatcgcac 300  
 tacggtagta actacgtaga ctactttgac tactggggcc aaggcaccac tgttacagtc 360  
 tcctctgac a 371

<210> 18  
 <211> 123  
 <212> PRT  
 <213> Chimaera sp.

<400> 18  
 Gln Val Gln Leu Val Ala Ser Gly Ala Glu Val Asn Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr  
 20 25 30

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Asn Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile  
 35 40 45

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe  
 50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Ser His Tyr Gly Ser Asn Tyr Val Asp Tyr Phe Asp Tyr Trp  
 100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Asp  
 115 120

<210> 19

<211> 114

<212> DNA

<213> Artificial Sequence

<220>

<223> N-template is a synthetic sense-strand oligonucleotide encoding a  
 amino acid 12-49 of the VH region (SEQ ID No. 18). The template i  
 s PCR-amplified by two primers (SEQ ID No. 20 and 21)

<220>

<221> V\_region

<222> (1)..(114)

<223>

<400> 19

aataagcctg gggcctcagt gaaggtctcc tgcaaggctt ctggctacac atttaccagt 60  
 tacaatatgc actgggtacg gcagcctcct ggaaggggccc tggaatggat tgga 114

<210> 20

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 1-19 of the VH region (SEQ ID No 18). The 3' end of the primer overlaps with the 5' end of the template by 24 nucleotides

<220>

<221> primer\_bind

<222> (1)..(57)

<223>

<400> 20  
caggtgcaac tggaggcttc cggggctgag gtaaataagc ctggggcctc agtgaag 57

<210> 21

<211> 55

<212> DNA

<213> Artificial Sequence

<220>

<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 43-60 of the VH region (SEQ ID No 18). The primer and the template overlaps by 21 nucleotides.

<220>

<221> primer\_bind

<222> (1)..(55)

<223>

<400> 21  
tgtaactagt atcaccattt cctggataaa tagctccaat ccattccagg cccct 55

<210> 22

<211> 126

<212> DNA

<213> Artificial Sequence

&lt;220&gt;

<223> C-terminal is a synthetic sense-strand oligonucleotide encoding a  
mino acid 70-111 of the VH region (SEQ ID No 18) The template is  
PCR-amplified by tow primers (SEQ ID No 23 and 24)

&lt;220&gt;

&lt;221&gt; V\_region

&lt;222&gt; (1)..(126)

&lt;223&gt;

&lt;400&gt; 22

ttgactgcag acaaatcctc cagcacagcc tacatgcagc tcagcagtct gacatctgag 60

gactctgcgg tctattactg tgcaagatcg cactacggta gtaactacgt agactacttt 120

gactac 126

&lt;210&gt; 23

&lt;211&gt; 61

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding am  
ino acid 57-76 of the VH region (SEQ ID No 18). The 3' end of th  
e primer overlaps with the 5'end of the template by 21 nucleotide  
s.

&lt;220&gt;

&lt;221&gt; primer\_bind

&lt;222&gt; (1)..(61)

&lt;223&gt;

&lt;400&gt; 23

tgatactagt tacaatcaga aattcaaggg caaggccaca ttgactgcag acaaatcctc 60

c 61

&lt;210&gt; 24

&lt;211&gt; 59

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 105-123 of the VH region (SEQ ID No 18). The primer and the template overlaps by 21 nucleotides.

&lt;220&gt;

&lt;221&gt; primer\_bind

&lt;222&gt; (1)..(59)

&lt;223&gt;

&lt;400&gt; 24

tgatcagagg agactgtaac agtgggtgcct tggccccagt agtcaaagta gtctacgta 59

&lt;210&gt; 25

&lt;211&gt; 321

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> FR-patched light chain variable region sequence (Full DNA Sequence) formed by joining the N- and C- terminal (SEQ 27 and 30) halves at the BspEI site.

&lt;220&gt;

&lt;221&gt; V\_region

&lt;222&gt; (1)..(321)

&lt;223&gt;

&lt;400&gt; 25

gatattcaac tcacacagtc tccatcaagt ctttctgcat ctgtggggga cagagtcaca 60

attacttgca gggccagctc aagtttaagt ttcatgcact ggtaccagca gaagccagga 120

tcctcccca aaccctggat ttatgccaca tccaacctgg cttccggagt ccctagtcgc 180

ttcagtggca gtgggtctgg gaccgagttc actctcaca tcagcagttt gcagcctgaa 240

gatttcgcca cttatttctg ccatcagtgg agtagtaacc cgctcacgtt cgggtgctggg 300

accaagctga ccgttctacg g

<210> 26

<211> 107

<212> PRT

<213> Chimaera sp.

<400> 26

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Leu Ser Phe Met  
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr  
35 40 45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu  
65 70 75 80

Asp Phe Ala Thr Tyr Phe Cys His Gln Trp Ser Ser Asn Pro Leu Thr  
85 90 95

Phe Gly Ala Gly Thr Lys Leu Thr Val Leu Arg  
100 105

<210> 27

<211> 129

<212> DNA

<213> Artificial Sequence

<220>

<223> N-template is a synthetic sense-strand oligonucleotide encoding amino acids 9-51 of the VL region (SEQ ID No. 26). The template is PCR-amplified by two primers (SEQ ID No. 28 and 29).

<220>

<221> V\_region

&lt;222&gt; (1)..(129)

&lt;223&gt;

&lt;400&gt; 27

tcaagtcttt ctgcatctgt gggggacaga gtcacaatta cttgcagggc cagctcaagt 60

ttaagtttca tgcactggta ccagcagaag ccaggatcct cccccaaacc ctggatttat 120

gccacatcc 129

&lt;210&gt; 28

&lt;211&gt; 45

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 1-15 of the VH region (SEQ ID No 26). The 3' end of the primer overlaps with the 5' end of the template by 21 nucleotides

&lt;220&gt;

&lt;221&gt; primer\_bind

&lt;222&gt; (1)..(45)

&lt;223&gt;

&lt;400&gt; 28

gatattcaac tcacacagtc tccatcaagt ctttctgcat ctgtg 45

&lt;210&gt; 29

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 45-57. The primer and the template overlaps by 21 nucleotides.

&lt;220&gt;



<221> primer\_bind

<222> (1)..(40)

<223>

<400> 29

ggactccgga agccagggtg gatgtggcat aaatccaggg

40

<210> 30

<211> 120

<212> DNA

<213> Artificial Sequence

<220>

<223> C-terminal is a synthetic sense-strand oligonucleotide encoding a  
mino acid 61-100 of the VH region (SEQ ID No 26) The template is  
PCR-amplified by tow primers (SEQ ID No 31 and 32)

<220>

<221> V\_region

<222> (1)..(120)

<223>

<400> 30

ttcagtggca gtgggtctgg gaccgagttc actctcacia tcagcagttt gcagcctgaa 60

gatttcgcca cttatttctg ccatcagtg agtagtaacc cgctcacgtt cggtgctggg 120

<210> 31

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding am  
ino acid 54-67 of the VH region (SEQ ID No 18). The 3' end of th  
e primer overlaps with the 5'end of the template by 21 nucleotide  
s.

<220>

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<221> primer\_bind

<222> (1)..(43)

<223>

<400> 31

ggcttccgga gtccttagtc gcttcagtgg cagtgggtct ggg

43

<210> 32

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 94-107 of the VH region (SEQ ID No 26). The primer and the template overlaps by 21 nucleotides.

<220>

<221> primer\_bind

<222> (1)..(42)

<223>

<400> 32

ccgtagaacg gtcagcttgg tcccagcacc gaacgtgagc gg

42